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Cage and flight pen evaluation of avian repellency and hazard associated with imidacloprid-treated rice seed

Michael L. Avery*, David G. Decker and David L. Fischer[†]
USDA/APHIS/Denver Wildlife Research Center, 2820 E. University Avenue, Gainesville, FL 32601, USA and [†]Miles Incorporated, 17745 South Metcalf Avenue, Stilwell, KS 66085, USA

A series of cage and flight pen trials evaluated the responses of male red-winged blackbirds (*Agelaius phoeniceus*) to rice seed treated with imidacloprid, a systemic insecticide. In two-cup trials, when both the treated and untreated seed was either dyed or undyed, individually caged redwings consistently avoided rice seed treated with 833 and 2500 mg kg^{-1} imidacloprid but not 278 mg kg^{-1}. When birds had a choice between undyed, untreated rice and dyed imidacloprid-treated rice, consumption of treated seed was suppressed at all levels. In a one-cup trial, rice consumption during the 4-day test phase was reduced relative to that during the 4-day pretest period at 833 and 2500 mg kg^{-1} of imidacloprid but not at 0 and 278 mg kg^{-1}. During 4-day trials in a 0.2 ha flight pen, six different ten-bird flocks removed an average of 41.1% of the untreated rice seed on sample quadrats compared with 8.8% lost from plots having 2500 mg kg^{-1} imidacloprid-treated seed. On the basis of residues from whole seeds and from hulls of rice seed eaten by test birds, it is estimated that redwings ingested 13–16% of the imidacloprid initially present on the seed. Thus, even at 2500 mg kg^{-1}, red-winged blackbirds feeding at an average rate of six seeds min^{-1} acquired only a fraction of the imidacloprid calculated to be a lethal dose. Imidacloprid appears to be an effective bird repellent seed treatment with minimal avian hazard.

Keywords: avian hazard; bird repellent; imidacloprid

Bird damage to newly planted crops, such as rice, is a major problem for growers in the southern United States (Wilson *et al.*, 1989; Decker, Avery and Way, 1990). Despite the identification of potentially useful bird-deterrent seed treatments (e.g. Daneke and Decker, 1988; Avery and Decker, 1992), commercial development of such new management tools requires a substantial financial commitment (Tobin and Dolbeer, 1987). These costs, and substantial regulatory expenses (Fagerstone, Bullard and Ramey, 1990), impose an economic burden that may be too high for application in most grain crops.

Compounds already marketed and registered for use against other types of agricultural pests may constitute a more economical source of bird damage control chemicals. For example, fungicides appear to have utility as bird-repellent seed treatments (Babu, 1988; Avery and Decker, 1991). Certain insecticides also seem to reduce depredating bird activity, either through direct repellency or mediated by a reduction in arthropod prey (Woronecki, Dolbeer and Stehn, 1981).

Imidacloprid, 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-*N*-nitro-1-*H*-imidazol-2-amine, is a nitromethylene heterocyclic systemic insecticide projected as a seed treatment on rice, cotton, wheat and other crops (Mullins, 1993). Initial feeding trials with red-winged blackbirds (*Agelaius phoeniceus*) and brown-headed cowbirds (*Molothrus ater*) suggested that imidacloprid is repellent to birds (Avery *et al.*, 1993). These results indicated that imidacloprid was not a sensory repellent like methyl anthranilate (Mason, Adams and Clark, 1989), but instead acted through postingestional distress (Rogers, 1978). Here, we report more detailed evaluations of the effectiveness of imidacloprid as a feeding deterrent on rice and we also assess the potential hazard posed by this compound to avian granivores.

Specifically, our objectives were as follows: (1) to measure consumption by singly caged red-winged blackbirds of rice seed treated with imidacloprid at various rates; (2) to determine whether incorporation of a dye in the seed treatment affected consumption of imidacloprid-treated rice; (3) to evaluate repellency of imidacloprid-treated rice to captive flocks of blackbirds; (4) to determine how much imidacloprid is removed from rice seed when blackbirds feed on it, and (5) to compare the birds' exposure to imidacloprid on rice seed to the acute lethal dose.

Materials and methods

Test food

Rice seed was treated with imidacloprid at four rates: 0, 278, 833 and 2500 mg kg^{-1}. The highest rate corresponded to the maximum projected recommended seed treatment rate (Mullins, 1993). Rice seed was treated

*To whom correspondence should be addressed

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and dyed by Gustafson, Inc., Dallas, Texas, USA and actual rates achieved were verified by an analytical laboratory (ABC Laboratories, Columbia, Missouri, USA) prior to testing. Additional seed samples were analysed (ABC Laboratories) after each of the cage tests to verify retention of chemical.

Testing procedure: general

Male red-winged blackbirds were trapped in Alachua County, Florida, USA, and held in captivity for 1–3 months prior to testing, during which time they had free access to F-R-M Game Bird Starter (Flint River Mills, Bainbridge, Georgia, USA) and water. Four days before the start of the pretest period, birds were removed from their communal holding cages, weighed, and assigned to individual test cages (45 × 45 × 45 cm) in an outdoor aviary. Test groups of eight birds each were formed by randomly assigning treatments to the cages. During the 4-day acclimatization period, the birds were provided with two clear-plastic food cups (8.2 cm diameter, 3.2 cm high, with a 3.1 cm opening in the top), each of which contained a mixture of untreated rice and Game Bird Starter.

Following acclimatization, there was a 4-day pretest period, a 2-day weekend break, and a 4-day test period. Throughout the pretest and test periods, Game Bird Starter was removed at 07:00, and 1 h later the test food cups were presented. After 3 h, the test food was removed and replaced with one cup of Game Bird Starter. Spilled rice seed collected on aluminium pans suspended beneath each cup was measured daily.

After each day's trial, we observed birds for indications of treatment-related effects such as vomiting or ataxia. After the study, all birds were weighed, banded, and released.

Two-cup trials

Three separate two-cup trials of imidacloprid-treated rice seed were conducted. In test 1, the treated and alternate seed were both undyed. In test 2, all of the seed was dyed. In test 3, the treated seed was dyed while the untreated alternate seed was not.

Prior to the pretest and test periods, one cup in each cage was assigned randomly as the treatment cup. Then, positions of treatment and alternate cups were switched daily. During pretest, both food cups contained 30 g untreated seed. In the test phase, the designated treatment cup contained imidacloprid-treated seed.

One-cup trial

During the 4-day pretest period, each bird received one cup of undyed, untreated rice. Then, on 4 test days, each bird was given one cup of dyed, imidacloprid-treated rice at its assigned rate. All other aspects of the procedure were as in the two-cup trials.

Analysis of cage trial results

For each bird, daily consumption was estimated by subtracting the mass of seeds remaining in each cup from the initial mass and then adding the mass of spilled seed. Each of the four cage feeding trials was analysed separately. Because no pretest differences in consumption were found among treatment rates or between cups (in the two-cup tests), the 4-day test period only was analysed in repeated-measures ANOVA with imidacloprid rate as the independent factor and day and cup as repeated factors. Tukey HSD test (Steel and Torrie, 1980) was used to isolate differences among means ($p < 0.05$). In each trial, changes in body mass from when the birds were placed in the test cages to when they were released were analysed in a one-way ANOVA.

Flight pen trials

Six week-long trials were conducted within the 0.2 ha flight pen at the Florida Field Station (Daneke and Avery, 1989). For each trial, eight 10 × 12 m plots were tilled and smoothed, and two plots (treatment and control) were randomly selected with the provision that there was at least 25 m separation between plots. The other six plots to be used in subsequent trials were covered with plastic tarps. On the treatment plot, 800 g rice seed treated with 2500 mg kg^{-1} imidacloprid were hand broadcast, and 800 g plain rice were hand broadcast on the control plot. Ten 0.19 m^2 sampling quadrats were randomly located within the treatment and control plots, and the seed count on each quadrat was adjusted to 50 to standardize initial densities and to facilitate subsequent counts. The seeds on the quadrats were re-counted at 24 h intervals to estimate the percentage of seed lost.

Three days prior to the start of each trial, ten male red-winged blackbirds were released into the flight pen and provided with a bowl of Game Bird Starter and rice. Each bird was weighed and fitted with a metal leg band attached to which was a uniquely numbered plastic tag.

Immediately after the rice was broadcast, and during 08:00–10:00 on each of the next three mornings, the redwings were observed from an elevated blind (hide) at the north end of the flight pen, and the duration and location (treatment plot or control plot) of each feeding bout was recorded. During each feeding bout, the number of rice seeds eaten by individual birds during 60 s segments was recorded. Each day, the 24-hour maximum and minimum temperatures and precipitation were noted from instruments mounted 1 m above the ground within the flight pen. At the end of each 4-day trial, the test birds were captured, reweighed, fitted with U.S. Fish and Wildlife Service bands, and released. Then the tarps were rearranged to cover used plots and expose two new plots in preparation for the next trial.

Results

Two-cup trial 1: all seed undyed

During the 4-day test period, total rice consumption did not differ ($p > 0.05$) among rates. Consumption from the treatment cup (1.01 g per bird) was lower ($p < 0.001$) than that from the alternate cup (1.97 g per bird). Rice consumption varied ($p = 0.045$) among days, being highest on day 3 (1.59 g per cup) and lowest on day 2 (1.38 g per cup).

Two- and three-way interactions were not significant, except for group × cup ($p < 0.001$). Blackbirds given seed treated at 0 and 278 mg kg^{-1} consumed seed equally from the two cups, whereas birds exposed to the 833 and 2500 mg kg^{-1} rates ate mostly ($p < 0.05$) from the alternate cup (*Figure 1A*).

Two-cup trial 2: all seed dyed

Total rice consumption during the 4-day test period varied ($p = 0.004$) among rates, decreasing from 2.03 g per cup at 0 mg kg^{-1} to 1.45 g per cup at 2500 mg kg^{-1}. Across all rates, mean consumption from the treatment cup (1.42 g per bird) was less ($p < 0.001$) than that from the alternate cup (2.08 g per bird). Consumption varied among days ($p < 0.001$), being least on day 2 (1.60 g per cup) and most on day 4 (1.95 g per cup). The significant ($p < 0.001$) group × cup interaction reflected the differences in consumption between cups at the 833 and 2500 mg kg^{-1} imidacloprid rates (*Figure 1B*).

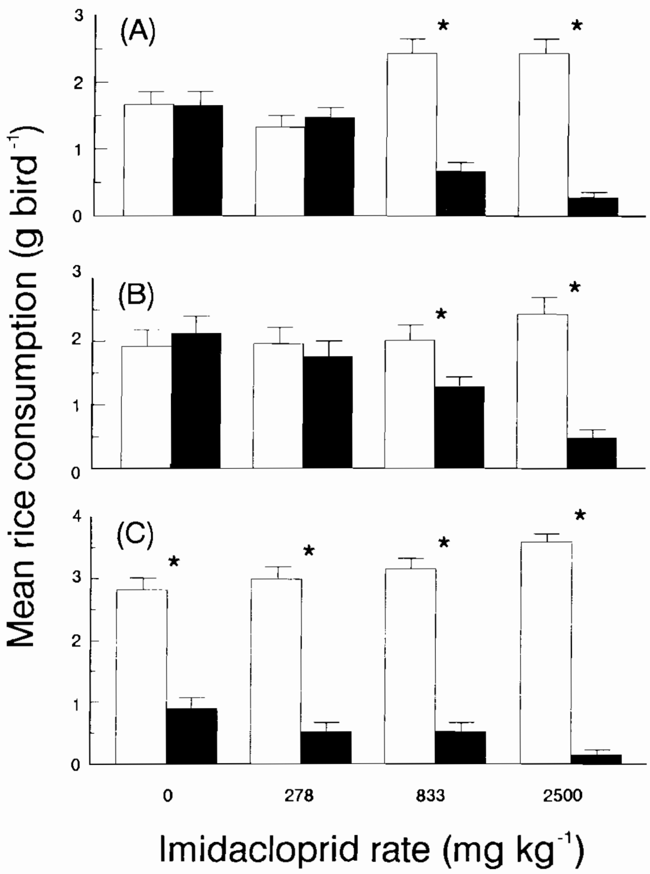

Figure 1. Mean rice seed consumption by male red-winged blackbirds exposed during 4-day feeding trials to (A) one cup of undyed, untreated rice seed and one cup of undyed, imidacloprid-treated rice seed, (B) one cup of dyed, untreated rice seed and one cup of dyed imidacloprid-treated rice seed, or (C) one cup of undyed, untreated rice seed and one cup of dyed, imidacloprid-treated rice seed. Asterisks denote significant ($p < 0.05$) difference in consumption between treated (solid bar) and untreated (open bar) rice. Capped bars denote 1 s.e.

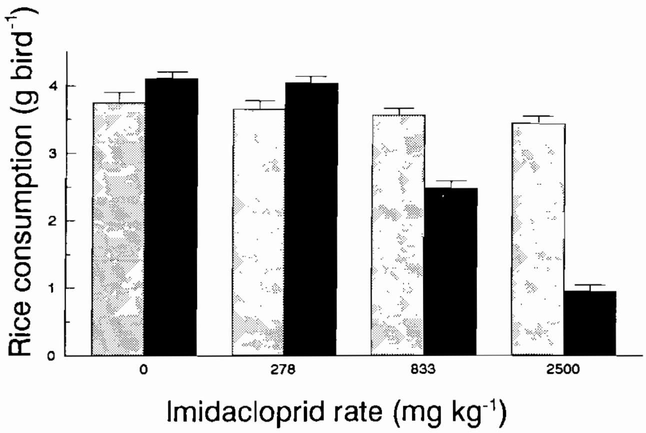

Figure 2. Mean rice seed consumption by male red-winged blackbirds exposed to one cup of undyed, untreated rice seed each for four daily pretest sessions (▯) and then one cup of dyed, imidacloprid-treated rice seed for four daily test sessions (■). Consumption differences were significant ($p < 0.05$) at 833 and 2500 mg kg^{-1}. Capped bars indicate 1 s.e.

Two-cup trial 3: treated seed dyed, alternate seed undyed

Total rice consumption during the 4-day test period did not differ ($p > 0.05$) among rates. Across all rates, consumption of treated rice (0.52 g per bird) was lower ($p < 0.001$) than that of untreated rice (3.15 g per bird). Rice consumption varied among days ($p = 0.001$), being greatest on day 3 (1.94 g per cup) and least on day 1 (1.74 g per cup). Rice consumption from the alternate cup exceeded ($p < 0.05$) that from the treatment cup at each imidacloprid rate (*Figure 1C*) and on each of the 4 test days.

One-cup trial: dyed seed

Overall, rice consumption varied ($p < 0.001$) among treatment rates, from 3.93 g per bird at 0 mg kg^{-1} to 2.20 g per bird at 2500 mg kg^{-1}. Consumption varied ($p < 0.001$) among days, increasing during the 4-day pretest period when untreated rice was presented, and then declining during the 4-day test period when treated rice was presented. The rate × day interaction ($p < 0.001$) indicated that consumption at the 0 and 278 mg kg^{-1} rates did not change from the pretest to the test period, whereas consumption at the 833 and 2500 mg kg^{-1} rates declined during the test period (*Figure 2*). Consumption was reduced by 1.08 g per bird (s.e. = 0.18 at the 833 mg kg^{-1} rate and by 2.49 g per bird (s.e. = 0.12) at 2500 mg kg^{-1}, compared with pretest values.

Flight pen trials

Across the six experimental trials, more seed ($p = 0.015$) was removed from the control plots than from the treatment plots. Overall, control plots averaged 41.1% (s.e. = 10.4%) seed loss compared with 8.8% (s.e. = 3.7%) in the treatment plots. Total seed loss did not vary ($p > 0.05$) across days. Although daily seed loss tended to decline in the control plot and increase in the treatment plot across the 4 days (*Figure 3*), the

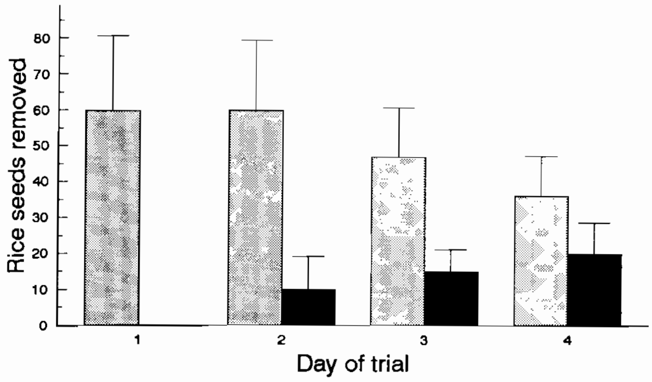

Figure 3. Mean daily number of rice seeds removed by ten-bird groups of male red-winged blackbirds from ten sampling quadrats in each of two experimental 9 × 12 m plots, one broadcast with untreated seed (□) and the other with rice treated with 2500 mg kg^{-1} imidacloprid (■). Test plots were >25 m apart within a 0.2 ha flight pen. Capped bars indicate 1 s.e.

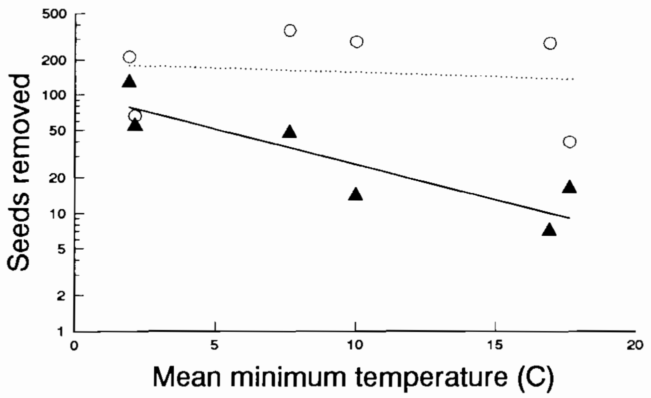

Figure 4. Relationship between the mean minimum temperature and the total number of seeds removed from ten sampling quadrats in 9 × 12 m treatment (▲——▲) and control plots (○. . . .○) during 4-day flight pen trials for each of six groups of ten male red-winged blackbirds. The treatment plot held rice seed treated with 2500 mg kg^{-1} imidacloprid; the control plot had untreated seed. Seed counts on all sampling quadrats were initially set at 50. The regression is of the form $Y = ae^{bx}$. For the treatment plots, a = 101.8, b = −0.14 and r^2 = 0.78. For the control plots, a = 184.8, b = −0.02 and r^2 = 0.02

interaction between plot and day was not significant (p = 0.083). Birds eating treated seeds did not react as if the seeds were distasteful or unpalatable.

Environmental conditions varied considerably during the six flight pen feeding trials. Among the six replicates, there appeared to be an inverse relationship between the number of treated seeds removed and the mean minimum temperature during the trial (*Figure 4*). No such relationship occurred with seed removed from the control plot.

During the study, we observed 18 individual birds that fed in both the control and the treatment plot. Across all 70 of these 1-min feeding rate observations, the 18 birds exhibited lower (p = 0.001) feeding rates in the treatment plot (5.4 seeds min^{-1}) than in the control plot (6.9 seeds min^{-1}).

There was no difference ($p > 0.05$) in initial mass of birds among the six trials. During the trials, however, the percentage of initial mass lost varied ($p < 0.001$). Mass loss was greatest in trials 3 (5.5%) and 4 (4.5%) and least during trials 5 (0.1%) and 6 (1.0%).

Imidacloprid residues

In each of the caged feeding trials, the residues on the seed samples approached the nominal values (*Table 1*). If the average seed mass is 27 mg, then the highest concentration tested (2500 mg kg^{-1}) amounted to a potential dose of 0.068 mg imidacloprid per seed. During the one-cup trial, we also sampled residues on the seed hulls. After adjusting for the ratio of hull mass to whole seed mass (1:5.6), we calculated that the hulls retained 84–87% of the initial imidacloprid concentration. Thus, at the 2500 mg kg^{-1} rate, redwings ingested only about 15% of the insecticide load, or roughly 0.0109 mg per seed (*Table 2*).

Discussion

In the two-cup cage trials, the redwings' tendency to eat imidacloprid-treated rice appeared to be related to their ability to distinguish between treated and untreated seed. When the two cups both held either dyed or undyed seed, the birds distinguished between treated and alternate seed only at 833 and 2500 mg kg^{-1}. Birds given dyed treated seed and dyed alternate seed (*Figure 1B*) appeared less able to discern the presence of imidacloprid than were those presented with only undyed seed (*Figure 1A*). We suspect that birds given undyed seed were able to tell treated from untreated seed by subtle differences in appearance imparted by the presence of the imidacloprid; at least, such differences were apparent to us. The birds

Table 1. Imidacloprid residues on rice seeds used in cage and feeding trials with captive male red-winged blackbirds

Nominal level (mg kg^{-1})	Residue measured (mg kg^{-1})[a] Trial 1	Trial 2	Trial 3	Trial 4
278	240	241	247	247
833	745	870	803	780
2500	2404	2468	2455	2482

[a]Measured by ABC Laboratories; recovery rate was 96% for trials 1 and 2, and 98% for the others

Table 2. Estimated ingestion of imidacloprid by red-winged blackbirds based on residues from whole rice seeds and from hulls only

Imidacloprid residue (mg kg^{-1}) Seed[a]	Hull[a]	Hull (adjusted)[b]	Quantity ingested by bird (mg per seed)[c]
247	1208	216	0.00084
780	3705	602	0.0032
2482	11646	2080	0.0109

[a]Measured by ABC Laboratories; [b]calculated by dividing full residue by 5.6, the ratio of whole seed mass to hull mass; [c]calculated by multiplying the hull-adjusted residue by 27 mg, the average seed mass, and dividing by 10^6

evidently relied upon visual rather than taste cues to detect the presence of the insecticide (Avery, 1984), and we hypothesize that the addition of the dye masked this visual difference between treated and untreated seed, causing poorer discrimination. The birds' ability to tell treated from untreated seed was further enhanced and consumption further suppressed in trial 3, when the treated seed was dyed but the alternate seed was not (*Figure 1C*).

Although the birds tended not to eat dyed seed in the presence of undyed alternate seed, they were not deterred by the dye itself. In the two-cup trial when all seed was dyed (*Figure 1B*) and in the one-cup trial (*Figure 2*), consumption of rice seed was unaffected by the dye alone. The results from trial 3 (*Figure 1C*) were due to a neophobic response in which birds preferred familiar, undyed seed to the different-looking dyed seed (Greig-Smith, 1987).

In the flight pen, the feeding behaviour of the birds appeared to be influenced by environmental conditions. We feel that the increase in treated seed consumption with colder temperature (*Figure 4*) indicated an increased food requirement due to harsher ambient conditions. Possibly, the availability of food in the control plot or surrounding grassy areas was insufficient to meet the elevated demands, and the birds turned to the treatment plot as an alternative. Our sampling of seed abundance did not indicate that seed in the control plot was drastically depleted, but our evaluation of the situation may not be the same as the birds' view of availability.

Predator activity may also have affected the birds' feeding behaviour. Sharp-shinned (*Accipiter striatus*) and red-shouldered hawks (*Buteo lineatus*) frequented the study site during flight pen trials 3 and 4, and often attacked the redwings even though they could not reach them. The redwings responded to this perceived threat by feeding in the plots closest to the cover, and in trials 3 and 4, these happened to be treatment plots. When forced to choose between feeding on untreated seed away from cover and treated seed close to cover, the birds opted for safety over palatability.

The finding that birds eating treated rice seeds ingested only about 15% of the insecticide load per seed helps explain why no deaths occurred. In the flight pen, the dose ingested per seed amounted to ~0.0109 mg. Feeding steadily at a rate of six seeds min^{-1}, a 60 g redwing would thus acquire a median lethal dose of imidacloprid in 38 min. This estimate is based on extrapolation of the median lethal dose of 41 mg kg^{-1} determined for the house sparrow (*Passer domesticus*; Mullins, 1993). In flight pen trials, the maximum length of time for which we observed a bird feeding at that rate in a treated plot was ~8 min. Thus, the birds appear to have a sizeable margin of safety between normal feeding rates and rates that would pose a high risk of lethality.

The observed difference in feeding rates of birds in the treated and control plots suggests that the treatment did affect the birds' feeding behaviour. Although we observed no ill effects in the flight pen trials, the slower feeding rate with the imidacloprid-treated seed may have been due to short-term inhibitory effects on the birds' nervous system known to be caused by nitro heterocyclic compounds (Taylor, 1990). During a previous study (Avery *et al.*, 1993), ataxia and temporary illness were observed in redwings and cowbirds that fed on imidacloprid-treated seeds. Nevertheless, present findings suggest that, at the proposed insecticidal levels, imidacloprid can be a safe, effective deterrent to birds feeding on rice seed. Additional evaluations, including field trials, of this compound as a bird deterrent are warranted.

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